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猪流感病毒 A/Swine/Inner Mongolia/547/01 分离株神经氨酸酶基因的克隆及真核表达

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摘要: 根据 GenBank 中登录的猪流感病毒参照序列设计了扩增神经氨酸酶(NA)基因的特异性引物。从猪流感病毒 A/Swine/Inner Mongolia/547/01(H3N2)株感染的鸡胚液中直接提取病毒 RNA,经 RT-PCR 扩增后将其克隆到 pMD18-T 载体上,进行序列测定和拼接。用 HindⅢ酶切后,亚克隆到杆状病毒转移载体 pMelBacB 上,得到重组转移载体 pMelBacNA,然后与线性化杆状病毒 DNA(Bac-N-Blue™DNA)共转染于 sf9 昆虫细胞。经 3 轮噬斑纯化,提取其 DNA 经 PCR 鉴定,获得重组杆状病毒株。SDS-PAGE 结果显示,目的基因已获得表达,Western blot 和神经氨酸酶活性检测结果显示表达产物具有良好的免疫原性和神经氨酸酶活性,为 SIV 亚单位疫苗和 NA 基因的深入研究奠定了基础。

关键词: 猪流感病毒; 神经氨酸酶基因; 克隆; 重组杆状病毒; 表达

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Cloning and expressing in the baculovirus expression system of NA gene of A/Swine/Inner Mongolia/547/01 isolate

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Abstract: We designed a pair of primers to amplify the NA gene of Swine Influenza Virus (SIV) A/Swine/Inner Mongolia/547/01 (SW/IM/547/01) based on the reported gene sequence. The complimentary DNA of NA gene was amplified by RT-PCR and was cloned into pMD18-T vector. The NA gene was subcloned into the transfer vector pMelBacB after being digested with HindⅢ. Then the purified pMelBacNA was co-transfected to log-phase sf9 insect cells with the linear DNA of baculovirus (Bac-N-Blue™ DNA) and Cellfectin reagent. The recombinant baculovirus were purified by three cycles of plaque assay with the chromogenic substrate x-gal in the agarose overlay. After that, the purified recombinant baculovirus was confirmed by PCR that it was not contaminated with field baculovirus. The results of SDS-PAGE indicated that NA gene was expressed successfully in sf9 insect cells. Western blot and NA assay indicated that the NA expressed by recombinant baculovirus in sf9 insect cells has good immunity and biological activity. The research is a good base for development of SIV subunit vaccine and the further study for the function of NA.

Key words: swine influenza virus; NA gene; cloning; recombinant baculovirus; expression

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猪流感(Swine Influenza, SI)是由猪流感病毒(Swin Influeoza Virus, SIV)引起的猪的一种急性、高度接触性呼吸道传染病,临床以突发、高热、咳嗽、流鼻涕、呼吸困难、高发病率、低死亡率为特征。该病发生后传播迅速,现地生产中极易继发感染猪呼吸-繁殖障碍综合征、猪呼吸道冠状病毒病、猪链球菌病等其它疫病,导致猪群料肉比下降,死淘率升高,对养猪业危害极大,是集约化养猪场普遍存在且难以根除的猪呼吸道病之一。近年,我国 SI 呈现不断蔓延的趋势,H3 亚型在全国范围内流行[1,2],猪群中还出现了 H5N1 和 H9N2

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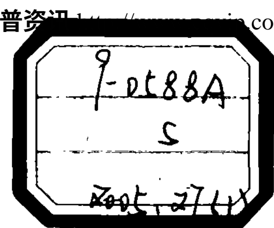

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3.3 测序结果在 www.flu.lanl.gov 网站 Blast 数据库中分析表明,与 A/Guangzhou/333/99(H9N2)和,A/Chicken/Hong Kong/G9/97(H9N2)NA 基因的核苷酸同源率最高,表明其遗传进化关系密切,值得进一步研究。

3.4 转染时重组转移载体质粒的纯度直接影响转染结果。如果质粒纯度低,将会含有大肠杆菌毒素及其它蛋白,这些污染物对 sf9 细胞有毒性,使细胞在转染后很快裂解死亡;再者质粒中的盐和其它带负电荷的粒子都会与带正电荷的脂质体相互作用而降低转染效率。这些都会导致重组病毒滴度降低或者根本没有重组病毒。本实验中选用上海华舜生物工程公司的小量质粒提取试剂,提取质粒后,又经酚/氯仿抽提,无水乙醇沉淀,干燥后,重悬于少量无菌去离子水中,转染效果很好。

3.5 本实验选取 Invitroge 公司的 pMelBacB 表达载体。该体系在杆状病毒 DNA 中引入 3 个 Bsu36I 酶切位点,这种修饰过的杆状病毒 DNA 经 Bsu36I 酶切后,得到的缺失复制必需基因 ORF1639 的线性化 DNA,即使自身环化也不能产生有复制能力的病毒。3个 Bsu36I 酶切位点的存在使得杆状病毒 DNA 一处也未切开的可能性很小,这在很大程度上避免 DNA 因未被酶切而造成的高野生病毒背景,从而重组病毒的比例可达 85 %~99 %。由于重组病毒含有基因可产生半乳糖苷酶分解底物而形成蓝色噬斑,而野生型病毒只能形成无色噬斑,因此重组病毒的筛选非常容易。

3.6 本试验选用带有蜜蜂蜂毒素信号肽的分泌型表达载体,但是用无血清培养基多次以感染重组杆状病毒的昆虫细胞培养上清为样品进行 SDS-PAGE,均没有见到预计大小的蛋白;然后将感染重组杆状病毒的昆虫细胞离心,用 PBS 洗涤 3 次后,再重悬于适量 PBS 中,超声波裂解后,进行 SDS-PAGE,可见到预计大小的蛋白。说明本试验构建的表达 NA 基因的重组杆状病毒未能将目的蛋白分泌到细胞培养上清中。未分泌的原因可能是因为目的蛋白较大,并且多角体基因启动子为极晚期启动子,待目的蛋白充分表达时,sf9 细胞中已有大量重组杆状病毒复制,sf9 细胞接近死亡导致运输蛋白的所需能量不足,而致使目的蛋白没有分泌出来。另外,也可能是对蜜蜂蜂毒素的信号肽和目的蛋白的基因分别进行转录、翻译,这样表达的目的蛋白因没有信号肽而无法分泌。

参考文献:

[1] 李海燕,于康震,辛晓光,等. 部分省市猪流感的血清学调查及猪流感病毒的分离与鉴定[J]. 动物医学进展,2003,24(3):67-72.

[2] 李海燕,李雁冰,于康震,等. 猪流感病毒的分离与鉴定[J]. 中国预防兽医学报,2002,24(1):12-17.

[3] 李海燕,于康震,杨焕良,等. 中国猪源 H5N1 和 H9N2 亚型流感病毒的分离鉴定[J]. 中国预防兽医学报,2004,26(1):1-6.

[4] Couch R B. Immunization for the prevention of influenza[M]. New York: Churchill Livingstone, 1989. 37-45.

[5] Compans R W. Influenza virus proteins analysis of polypeptides of the virion and identification of spike glycoproteins[J]. Virology, 1970, 42: 880-889.

[6] 萨姆布鲁克, E F 弗里奇, T 曼尼阿蒂斯. 分子克隆实验指南[M]. 第2版,金冬雁,黎孟枫,等译. 北京:科学出版社,1996.

[7] Stefen Gruenwald M D, Judi Heitz M S. Baculovirus expression vector system: procedures and methods manual[M]. Pharmingen, 1993, 9-10.